WS10.1 The performance of the UK national newborn screening (NBS) programme for CF – Results from a UK regional paediatric network

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Objectives: Newborn screening (NBS) for cystic fibrosis (CF) began in Merseyside and Cheshire in 2007. The UK protocol is IRT-DNA-IRT with a cut-off of 99.5% for IRT-1. The DNA panel covers the 4 commonest UK mutations. For infants with 1 mutation a second IRT is measured at day 21 of life. In addition, infants with no mutations but an IRT-1 >99.9th centile also have IRT-2 at day 21. There is interest in the impact of these unique aspects of the UK protocol, and we have reviewed our results to assess the sensitivity of the programme.
Methods: We reviewed the performance of the NBS programme in our UK region matching data from the one screening laboratory with clinical data from the network.
Conclusion: 172,989 infants have been screened in our region since 2007. Of 95 with a "CF suspected" NBS result, 81 were diagnosed with CF (PPV, 0.85). 5 infants with meconium ileus had false negative NBS results with a low IRT-1, but with no delayed diagnosis. 4 infants had a delayed diagnosis due to a false negative NBS result (mean age at diagnosis, 10 months). Of these, 3 infants had IRT-1 values below threshold, and no further testing undertaken. In the 4th infant, the 4-mutation panel was negative and IRT-1 <99.9th centile. Excluding the infants with meconium ileus the sensitivity of the protocol is 96%.
The UK protocol was designed to reduce unnecessary stress to families and our data suggests this has been achieved. Sensitivity is adequate by international standards. 3/4 false negative results were from a low IRT-1. The case missed by the 4 mutation panel raises concerns about whether the benefits of limiting the initial panel are outweighed by a reduction in sensitivity.

WS10.2 Complete sequencing of 2,000 cystic fibrosis and CFTR-related disease high risk alleles

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Since 2007, we have fully sequenced over 2,000 CF and CFTR-Related Disease alleles. We have employed fully automated bi-directional fluorescent Sanger sequencing on samples referred mainly as DNA samples from other regional laboratories. MLPA (MRC Holland) has also been employed to detect larger deletions or duplications. Although most referrals were seeking a diagnosis of cystic fibrosis, a growing number have been for CFTR-Related Diseases, including bronchiectasis, CBAVD and pancreatitis. Samples from other laboratories had either one or no mutations already identified (80% and 20% respectively). Mutations were identified in 61% of alleles overall, comprising 175 different CF mutations, of which 25 were previously undescribed. Twenty-eight cases were found to have large deletions (13 different mutations in 25 alleles) or duplications (2 in 3 alleles). We have also identified a large number of variants of uncertain clinical significance, especially in the CFTR-RD cohort. Patients originating from the Indian Subcontinent demonstrate a high degree of consanguinity and subsequent homozygosity for rare mutations not typically seen in the UK native population.
Complete sequencing and MLPA analysis of high risk CF alleles shows up many different types of mutations, including larger deletions and duplications, and variations of uncertain clinical significance. With the increasing number of referrals for CFTR-RD, the assessment of novel sequence changes is becoming more important. A number of cases remain with a strong clinical suspicion, but without two convincing mutations; in such cases, mRNA analysis is indicated, which may reveal the presence of deep intron mutations.

WS10.3 What can next-generation sequencing do for CF?

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Objectives: Recent technological advances in the next-generation sequencing (NGS) offer the possibility of re-sequencing analysis of DNA from CF patients in which only one mutation has been detected. Using two different strategies for whole *CFTR* gene (253 kb) sequencing, we report our first findings in underexplored *CFTR* gene regions (5′ and 3′ uncoding regions and deep intronic regions) not routinely analyzed by current scanning methods (mutation detection rate: 95 to 99%).
Methods: Using GS Junior platform (Roche, 70 Mb per run), the two strategies evaluated consist in a targeted sequencing capture (SeqCap EZ Choice Library System, NimbleGen) and a pre-amplification system by Long Range.
Results: On average 110 sequences variations were detected per DNA. Using exclusion filters (heterozygosity, SNPs included in databases), we selected 4 variations per DNA in the large deep intronic sequences. After *in silico* analysis, two mutations identified in two different patients were studied by functional *in vitro* approaches (minigene assay) that led to identify new aberrant splicing events.
Conclusion: Identification and characterization of mutations in previously unexplored regions is a real advance in CF diagnosis and has implication in genetic counseling for patients and families. Moreover, determination of new mutations offers a highlight for molecular mechanisms that govern transcription and maturation of the *CFTR* gene expression.
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WS10.4 A new molecular strategy using CFTR2 data for improving IRT/DNA screening

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Objectives: Newborn screening (NBS) for CF is ongoing throughout the USA, and most states analyze 23–40 *CFTR* mutations when IRT levels are high (2-tier IRT/DNA algorithm). Infants with 1 mutation are usually referred for sweat testing but ~90% have normal chloride levels and thus are false positive screens. To improve NBS, we are evaluating expanded DNA analyses after a single mutation is detected using a 3rd tier panel of the 157 mutations identified in the CFTR2 project.
Methods: MiSeqDx™ Cystic Fibrosis Solution, a genotyping-by-sequencing next generation assay, is being used to detect 157 *CFTR* mutations that are CF-causing alleles. Initially, for method validation, specimens from 45 Wisconsin newborns were evaluated with 2 known *CFTR* mutations. Mutations identified by MiSeqDx™ are confirmed by Sanger sequencing.
Conclusion: Validating MiSeqDx™ Cystic Fibrosis Solution for its analytic sensitivity and specificity using DNA isolated from NBS dried blood specimens, we found 100% concordance in detecting 90 *CFTR* alleles. Our experience thus far demonstrates the feasibility of using next generation sequencing in a public health laboratory, and establishes the foundation for the further study of a 3-tier IRT/DNA/DNA screening protocol in reducing false positive results caused by CF heterozygote carrier infants. In April, we plan on extending the study to states included in the newly established Great Lakes Consortium (IL, IN, MI, MN, and WI) which collectively screen more than 500,000 newborns annually. If this 3-tier method is validated and cost effective, we envision that only newborns with 2 CF-causing mutations might be reported as positive.